Favorable reconsideration and withdrawal of the objections and rejections of the subject application are respectfully requested in view of the above changes and the following remarks.

This Amendment is being submitted to present the claims in a better form and to address the rejections set forth in the official action.

**Response to Rejections under 35 USC 112**

Claims 129 and 134-137 are rejected under 35 USC 112, second paragraph. Claims 129 and 134-137 have been amended to address the rejections under 35 USC 112.

"M" is now defined in claim 129, part (a) as an integer greater than 1. Claim 129, part (c), has been amended to more particularly point out and define that the tag attaches to the solid support. Claim 129, parts (f) and (g), have been amended to address the Section 112 rejections directed thereto. Antecedent basis has been provided in claim 129 for the recitation of "fluorophore tags" in the dependent claims.

Reconsideration and withdrawal of the Section 112 rejections is respectfully requested.

**Response to Rejections under 35 USC 102 and 103**

The Invention

The claimed invention provides a method for identifying a compound of interest in a library of compounds by placing one or more tags (each tag is a fluorophore or chromophore) on each of a multiplicity of solid supports (typically beads) during each coupling step (see claim 1 (c)) in the course of "Divide, Couple and Recombine" (DCR) combinatorial synthesis. The chemical identity of the compound synthesized and displayed on each bead is thus uniquely encoded. Each tag is optically distinguishable by wavelength, excited-state lifetime or emission

intensity. An assay is performed on the library of bead-bound compounds for a property of interest (Specification, page 16, line 24+, describing an assay method). Decoding of bead identity, and hence the identity of the attached compound having the property of interest, is based on the determination of the relative abundance of one or more tags on each bead of interest by in-situ optical interrogation (Specification, page 9, lines 14-21; page 17, line 29 - page 18, line 2; page 20, lines 6-27). In-situ optical interrogation determines the code of each bead without isolating the bead from other beads, or from the configuration in which beads are assayed for a property of interest. In-situ optical interrogation provides the advantage that the tags need not be cleaved from the beads, or otherwise modified prior to or in the course of the decoding process.

Fig.7 is an illustration of the in-situ method of interrogation and decoding of the invention. A preferred format for in-situ interrogation and decoding involves planar arrays of beads. Each constituent bead displays a compound, as well as tags. Automated screening assays to identify compounds of interest are performed without the time-consuming and error-prone steps of bead physical separation or isolation (such as by flow cytometry) and chemical cleavage of tags. The in-situ optical interrogation of the present invention is more efficient in terms of the time and labor generally required for the decoding of a compound of interest in a chemical library (Specification pages 3 and 4). Another advantage is the ability to miniaturize the process by using small synthesis beads (Specification, page 21, lines 8-24).

The present invention takes advantage of a property of the tags, namely that they are optically distinguishable by wavelength, excited-state lifetime or emission intensity. An emission spectrum is collected and recorded from which relative abundances of the tags on each bead are determined and the color code deciphered (Specification page 10, lines 4-13). Based on

these optical characteristics, one is able to construct unique chemical codes, for example, in the form of a binary or an extended binary color code comprised of one or more tags.

Binary or extended binary color codes offer large coding capacities and provide an efficient strategy for encoding a smaller set of distinct beads. Each tag represents one bit, and a set of tags on a bead represents a set of bits. Each tag is uniquely associated with a specific reaction step and component on a bead. An alphabet of codes is uniquely associated with a specific reaction sequence and a corresponding compound synthesized and displayed on a bead.

For example, by way of illustration of a binary code, without any intention of limiting the scope of the invention, a reaction sequence with two components, *a* and *b*, is encoded by zero or one tag as follows, wherein *d* represents one bit:

| | *d* |
|---|---|
| *a* = 1 unit of d; | 1 |
| *b* = 0 unit of d; | 0 |

A reaction sequence with four components, *a-d*, could be encoded by zero, one or two tags as follows, wherein $d_1$ and $d_2$ represent two bits:

| | $d_1 d_2$ |
|---|---|
| *a* = 1 unit of $d_1$, 1 unit of $d_2$; | 1 1 |
| *b* = 1 unit of $d_1$, 0 unit of $d_2$; | 1 0 |
| *c* = 0 unit of $d_1$, 1 unit of $d_2$; | 0 1 |
| *d* = 0 units of $d_1$, 0 units of $d_2$; | 0 0 |

A reaction sequence with eight components, *a-h*, could be encoded by zero, one, two, three tags as follows, wherein $d_1$-$d_3$ represent three bits:

| | $d_1 d_2 d_3$ |
|---|---|
| a = 1 unit of $d_1$, 1 unit of $d_2$, 1 unit of $d_3$; | 1 1 1 |
| b = 1 unit of $d_1$, 1 unit of $d_2$, 0 unit of $d_3$; | 1 1 0 |
| c = 1 unit of $d_1$, 0 unit of $d_2$, 1 unit of $d_3$; | 1 0 1 |
| d = 0 unit of $d_1$, 1 unit of $d_2$, 1 unit of $d_3$; | 0 1 1 |
| e = 1 unit of $d_1$, 0 unit of $d_2$, 0 unit of $d_3$; | 1 0 0 |
| f = 0 unit of $d_1$, 0 unit of $d_2$, 1 unit of $d_3$; | 0 0 1 |
| g = 0 unit of $d_1$, 1 unit of $d_2$, 0 unit of $d_3$; | 0 1 0 |

h = 0 unit of $d_1$, 0 unit of $d_2$, 0 unit of $d_3$. 0 0 0

(See specification page 10, line 15 to page 14, line 15).

The in-situ optical interrogation approach of the invention represents an advance over the prior art and solves the problem of the general scattering phenomena expected for spectral analysis performed in heterogeneous media. The latter tends to diminish spectral signals hindering accurate determination of tag relative abundance information (Specification page 5, line 30 to page 6, line 6). The present method also avoids fluorescence energy transfer between different dyes (Specification, page 15, line 16). The invention provides for color-encoded amino acid and peptide combinatorial libraries, or any other precursors and compound classes created via DCR combinatorial synthesis.

Response to Rejections

Claims 129-138, 142-146, 151, 154 and 159 are rejected under 35 USC 102(b) as being anticipated by WO 93/06121 (Dower). Claims 129-138, 142-146, 151, and 155-159 are rejected under 35 USC 102(e) as being anticipated by U.S. Patent No. 5,968,736 (Still). Reconsideration is respectfully requested.

"For a prior art reference to anticipate in terms of 35 USC 102, every element of the claimed invention must be identically shown in a single reference." In re Bond, 15 USPQ2d 1566, 1567 (Fed. Cir 1990). MPEP 2131. For the following reasons the claimed invention is not anticipated by Dower or Still.

Dower

Dower teaches a method of synthesizing diverse collections of random oligomers using identification tags on the oligomers to facilitate identification of oligomers with desired properties.

The identifier tags are directly attached to the oligomer with or without an accompanying particle, or attached to a solid support (e.g., bead) upon which the oligomer is synthesized. The synthetic oligomers (i.e., the compounds of interest) thus displayed are screened for the ability to bind to a receptor (p.29, line 35 - p. 30, line 2). In the next step, the individual beads displaying the desired oligomer ligand which binds to the probe receptor are then ***selected and sorted*** from the bead library (p. 30, lines 5-12). For example, positive beads are isolated using fluorescence activated cell sorting (FACS), where the receptor used to probe the library is fluorescently labeled. Alternatively, the positive beads are isolated by affinity adsorption (p. 30, lines 13-25).

In another embodiment, Dower teaches the generation of a library of soluble tagged oligomers. Microscopic beads are placed in individual wells. The oligomers are cleaved from the beads and remain contained within the compartment along with the bead and the attached identifier. The individual bead wells are screened with a probe ligand, which may be labeled by e.g., fluorescence. Beads are isolated from the positive wells by micromanipulator plucking or FACS. The tag, which in this case is an oligomer (not an optically distinguishable tag as claimed by Applicants), is then decoded by amplification and sequencing (p. 30, line 26 - p.31, line).

In the principal embodiment of Dower, the identifier tags are oligonucleotides. Decoding is by determination of specific nucleotide sequence. The "tag", which is a sequence of nucleotides, is not an optical tag, and is not optically decoded. Rather, the oligonucleotide tag is decoded by hybridization to one or more further labeled oligomers, which is a chemical, not optical, decoding process. Multiple such rounds of chemical modifications (i.e., hybridizations) must be performed to permit interrogation of a multiplicity of identifier tags. This is an arduous and lengthy procedure which is obviated by the present invention.

In another embodiment, Dower disclosed identifier tags composed of a set of light-addressable fluorophores. The light-addressable fluorophores are fluorescent or phosphorescent moieties incorporated into the beads prior to the synthesis of the oligomers of the oligomer library. The spectral properties of the light-addressable compounds can be changed so as to store information. A bead may incorporate a variety of fluorophores, each of which must be selectively photo-bleached and so rendered incapable of fluorescence, or rendered of diminished fluorescence (p. 20, l. 24-32). However, Dower teaches, as with oligonucleotide-tagged beads, that light-addressable fluorophore-tagged beads are isolated prior to tag identification. This is contrary to the claimed invention, which provides for *in situ* optical interrogation of beads without isolation from other beads in the array, or from the configuration in which beads are assayed for a property of interest.

In each case described by Dower, the desired beads are first isolated and separated from other beads before the tag is decoded to ascertain the sequence of the oligomer on the bead (p. 26, lines 16-17). Dower does not teach a method in which an *optically distinguishable tag* attached to a solid support is decoded by ***in situ optical interrogation without isolating the solid support of interest from other solid supports***.

Still

Still discloses methods for recording the reaction history of a ligand synthesized on a solid support using organic molecules as tags to produce a binary code defining choice of reactant, stage of reaction, etc. After the synthesis is completed, the reaction products, referred to as "ligands" by Still, are screened for a desired property either after detachment from the bead or while still attached. Beads with attached ligands are incubated in aqueous buffer with fluorescently-labeled monoclonal antibody (to determine the presence of the desired property -

here, binding to the MAb). The "positive" fluorescing beads are separated manually or by means of FACS from the "negative" non-fluorescent beads, to the extent the tags are retained on the bead under the conditions of sorting. Each selected fluorescent bead is subjected to a means for releasing at least some of the tags from the bead for purposes of decoding (Column 17, lines 4-18).

Still does not anticipate Applicants' invention, because nowhere does Still teach or suggest tag decoding ***without isolating the solid support of interest from other solid supports.*** In fact, Still requires separation of beads containing attached fluorescent-MAb by means of FACS, to the extent tags are retained on the bead under the condition of sorting.

Still does not anticipate the claimed invention for a further reason. Following the bead sorting and isolation step, each selected fluorescent bead is subjected to a means for releasing at least some of the tags from the bead (Column 17, lines 10-18). Thus, Still teaches away from the feature of the claimed invention wherein decoding takes place ***without detaching any of the tag(s)from the solid support.***

The requirement of tag detachment from the solid support is apparent from the disclosure of Still. Decoding, for purposes of compound identification, is carried out by isolating the beads of interest from other beads, and by cleaving the tags from the beads for further off-line analysis (column 6, lines 26 to 41). The tag is attached to the support by a *cleavable* linker, such that the tag may be released from the solid support by cleaving the linker (column 4, lines 8-25). While the ligand (i.e., the synthesized compound) may remain attached to the bead for purposed of screening for a property of interest, the tag is detached from the bead to allow decoding.

The detachable tags of Still are amenable to rapid analysis by an automated sampling system. Such detachable tags allow for selective derivatization to facilitate detection via

functional groups, eliminating any incompatibility between the detection moiety and the reaction conditions used in the synthesis. By contrast, the methods of Applicants' invention are premised on the use of tags which remain attached to the solid support, unlike the detachable tags of Still.

Applicants respectfully submit that neither Dower nor Still anticipate the claims of the present application. Reconsideration and withdrawal of the Section 102 rejections is earnestly solicited.

**Rejection under 35 USC 103(a)**

Claims 129-159 are rejected under 35 USC 103 as being unpatentable over Dower in view of U. S. Patent No 5,728,529 (Metzker).

The Examiner states that it would have been obvious to a person skilled in the art at the time the invention was made to use the fluorescent dyes taught by Metzker as identifier tags in the method of oligomer library synthesis and identification of Dower.

Metzker fails to remedy the deficiencies of Dower discussed above. Specifically, Metzker fails to teach or suggest a method of identifying compounds of interest in a library of compounds bound to solid supports wherein an optically distinguishable tag on a solid support of interest (carrying a compound of interest) is interrogated in situ, without isolation from other solid supports. Even if Dower and Metzker are properly combinable, which is not admitted, the resultant is not the claimed invention.

Reconsideration and withdrawal of the Section 103 rejection is respectfully requested.

## Conclusion

The claims remaining in the application are believed in condition for allowance and an early notification of such is solicited.

Respectfully submitted,

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BY: _____

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## Appendix A

129. (Amended) A method of identifying a compound of interest in a library of compounds, each of said compounds being bound to a solid support and being produced by a unique reaction series composed of N reaction steps, wherein N is an integer of at least 2, and wherein each compound is produced from components which are independently the same or different, the method comprising:

(h) [Dividing] dividing a population of solid support into M batches, wherein M is an integer greater than 1;

(i) [Reacting] reacting each of the M batches of solid support with a component, so that the component forms a bond with the solid support;

(c) adding to [each batch] one or more batches, prior to (b), concurrently with (b), or subsequently to (b), one or more tag(s), each tag able to be attached to the solid support and able to be identified by optical interrogation, wherein said one or more tag(s) constitutes a code, which code is uniquely associated with a compound and a corresponding reaction sequence and is determined by optical interrogation; [a spectrally distinguishable fluorophore tag uniquely associated with each component and reaction step, said fluorophore tag being capable of forming a bond to a solid support, wherein said fluorophore tag represents one or more bits of binary code and comprises zero, one, or more than one fluorescent dye(s), said dye(s) being spectrally distinguishable by wavelength, excited-state lifetime or emission intensity;]

(d) recombining all of said M batches after (b) and (c);

(e) repeating (a) to (d) for N-1 times, or repeating (a) to (d) for N-2 times followed by repeating (a) to (c) once, to produce a library of compounds;

(f) performing an assay capable of indicating that any compound in the library has a property of interest; and [contacting the library of compounds with a target biomolecule, wherein the target biomolecule is capable of binding to a compound in the library having a property of interest; and]

(g) decoding the code composed of one or more tag(s) [the fluorophore tag(s) associated with the compound having the property of interest] to identify the [said] compound associated with the code, wherein the decoding step is carried out without isolating the solid support of interest from other solid supports and without detaching any of the tags(s) from the solid support of interest and wherein said decoding step comprises in-situ optical interrogation of the tag(s). [optically interrogating the fluorophore tag(s) bound to the solid support on which the compound having the property of interest was produced.]

130. (Amended) The method of claim 160 [129] wherein the solid support comprises a bead.

131. (amended) The method of claim 160 [129] wherein (c) comprises repeating (a) to (d) for N-1 times to produce a library of compounds.

132. (Amended) The method of claim 160 [29], wherein (e) comprises repeating (a) to (d) N-2 times followed by repeating (a) to (c) once to produce a library of compounds.

134. (Amended) The method of claim 160 [129], wherein each [the] fluorophore tag [tags added in (c)] is [are] in substoichiometric amount compared to the component [components] added in (b).

135. (Amended) The method of claim 160 [129], wherein each [the] fluorophore tag [tags] added in (c) is [are] from about 0.001 to about 0.1 molar equivalent to the component [components] added in (b).

136. (Amended) The method of claim 160 [129], wherein the optical interrogation of each [the] fluorophore tag [tags] comprises determining its relative abundance. [the value of each of the constituent fluorescent dyes.]

137. (Amended) The method of claims 160 [129], wherein each [the] fluorophore tag [tags] is [are] attached to the solid support [supports] by covalent bonding.

138. (Amended) The method of claim 160 [129], wherein the fluorophore tag is capable of forming a bond to the solid support directly or to the component attached to said solid support.

139 (Amended) The method of claim 160 [129], wherein the fluorophore tag [the fluorescent dyes] is a dye [comprises dyes] selected from the group consisting of compounds with the following chemical structures [names]:

3-(ε-carboxypentyl)-3'-ethyl-oxacarbocyanine-6,6'-disulfonic acid,

1-(ε-carboxypentyl)-1'-ethyl-3,3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonic acid,

1-(ε-carboxypentyl)-1'-ethyl-3,3,3'3'-tetramethyl-3H-benz(e)indocarbocyanine-5,5',7,7'-tetrasulfonic acid, and

1-(ε-carboxypentyl)-1'-ethyl-3,3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonic acid, and [are] is activated as an active ester[s] selected from the group consisting of succinimidyl, sulfosuccinimidyl, p-nitrophenol, pentafluorophenol, HOBt and N-hydroxypiperidyl.

140. (Amended) The method of claim 160 [129], wherein the fluorophore tag [the fluorescent dyes] is a dye [comprises dyes] selected from the group consisting of compounds with the following chemical structures [names]:

6-((4,4-difluoro-5,7-dimethyl- 4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino) hexanoic acid,

6-((4,4-difluoro-5-phenyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino) hexanoic acid,

6-((4,4-difluoro-1,3-dimethyl-5-(4-methoxyphenyl)-4-bora-3a,4a-diaza-s-indacene- 2-propionyl) amino)hexanoic acid,

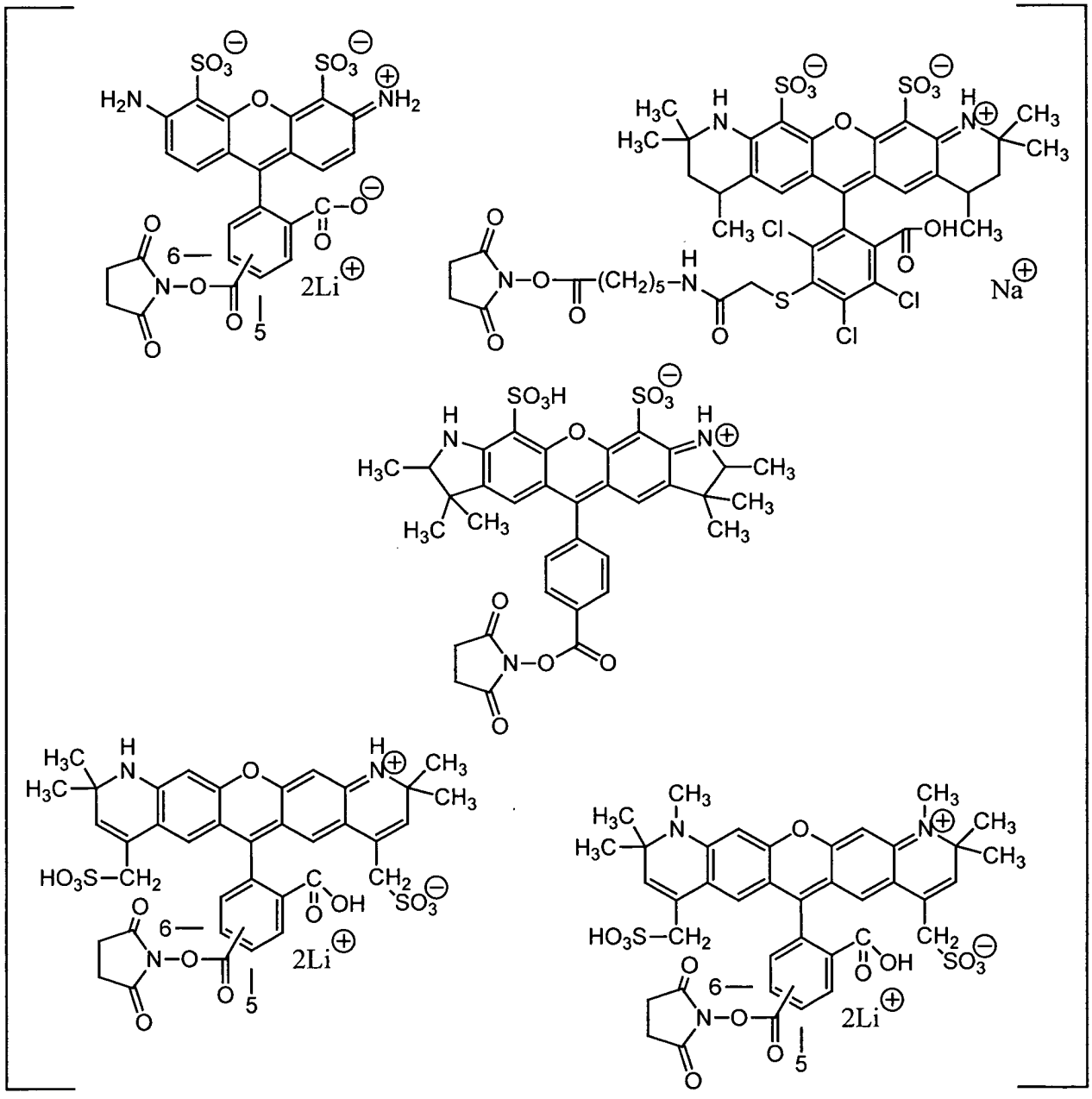
6-(((4-(4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)phenoxy) acetyl) amino)hexanoic acid,

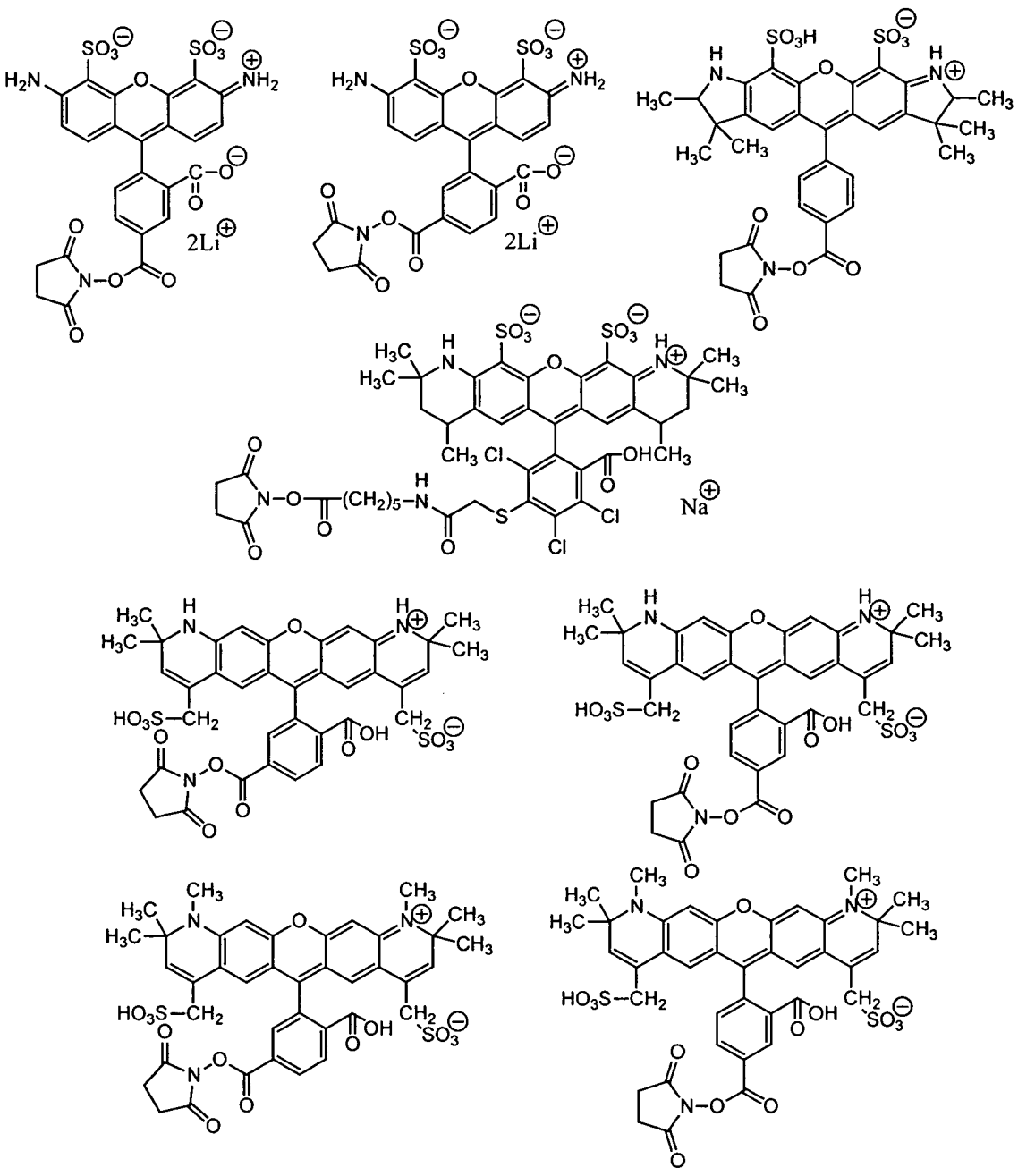
6-(((4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)styryloxy)acetyl) aminohexanoic acid, and

6-(((4,4-difluoro-5-(2-pyrrolyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)styryloxy) acetyl)aminohexanoic acid,

and [are] is activated as an active ester[s] selected from the group consisting of succinimidyl, sulfosuccinimidyl, p-nitrophenol, pentafluorophenol, HOBt and N-hydroxypiperidyl.

141. (Amended) The method of claim 160 [129], wherein the fluorophore tag [the fluorescent dyes] is a dye [comprises dyes] selected from the group consisting of compounds with the following chemical structures:





142. (Amended) The method of claim 160 [129], wherein (g) is carried out using multi-color [fluorescent] fluorescence imaging or spectral imaging analysis.

143. (Amended) The method of claim 160 [129], wherein the decoding is carried using multi-color [fluorescent] fluorescence imaging in combination with spectral analysis.

144. (Amended) The method of claim 160 [129], wherein M is an integer from at least 2 to 25.

145. (Amended) The method of claim 160 [129], wherein the component is protected or unprotected at a group which is capable of participating in a further coupling reaction and orthogonally protected at non-participating group(s), and wherein (d) further comprises cleaving any protecting group of the component which is to participate in a further coupling reaction.

146. (Amended) The method of claim 160 [129], wherein the fluorophore tag [fluorescent dyes] is [are spectrally] optically distinguishable by emission wavelength.

147. (Amended) The method of claim 160 [129],wherein the fluorophore tag [fluorescent dyes] is optically [are spectrally] distinguishable by emission intensity[, the emission intensity being distinguishable] by adjusting the ratio [ratios] of the relative quantities of the [each] fluorophore tags [dye].

148. (Amended) The method of claim 147, wherein [the fluorophore tag comprises two fluorescent dyes,] the ratio [of said dyes being] is from about 1:1 to 4:1.

149. (Amended) The method of claim 160 [129], wherein the fluorophore tag [fluorescent dyes] is optically [are spectrally] distinguishable by excited-state lifetime.

150. (Amended) The method of claim 160 [129], wherein the fluorophore tag [fluorescent dyes] is optically [are spectrally] distinguishable by emission wavelength, excited-state lifetime and emission intensity.

151. (Amended) The method of claim 160 [129], wherein the compound of interest comprises an oligonucleotide or nucleic acid.

154. (Amended) The method of claim 160 [129], wherein N is an integer from at least 4 to about 12.